J. Fac. Agric. Iwate Univ. 22 (1) : 25-30 (1994)

Immunoblot Analysis of Hydatid Cyst Fluid Antigens Using Sera of Unilocular Hydatidosis in Cattle and Sheep

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(Received on May 24, 1994)

Tadashi ITAGAKI, Tsukasa SAKAMOTO, Patricia BERASAIN, Jaqueline MAISONNAVE and Luis YARZÃBAL (1994) **Immunoblot Analysis of Hydatid Cyst Fluid Antigens Using Sera of Unilocular Hydatidosis in Cattle and Sheep. J. Fac Agric. Iwate Univ. 22 : 25-30.** Hydatid cyst fluids from cattle (HCFC) and sheep (HCFS) were separated by SDS-PAGE and assayed in immunoblots for reaction with sera of unilocular hydatidosis in cattle and sheep. The composition of dominant polypeptides between HCFC and HCFS differed from each other. Polypeptides of 21 kDa, 14 kDa and 8 kDa in HCFC, and of 50 kDa, 27 kDa and 14 kDa in HCFS were recognized by most of sera of unilocular hydatidosis in sheep and cattle, respectively, but also by a few negative sera. These 5 polypeptides seemed to be corresponded to subunits of Antigen B which is one of major antigens in hydatid cyst fluid.

Key words: domestic animals, *Echinococcus granulosus*, hydatid cyst fluid antigen, immunoblot, unilocular hydatidosis.

I. Introduction

Unilocular hydatidosis caused by larvae of *Echinococcus granulosus* is mainly the disease of domestic animals and human. A variety of serological tests have been carried out to diagnose the disease in domestic animals [1, 3, 6, 8, 11, 13, 15]. However, there are few serological tests which have been applied in practical diagnosis, because most of the tests gave rise to false negative and false positive reactions. These false reactions are thought to result from the poor productivity of anti-*E. granulosus* antibody in naturally infected animals [1, 6, 8, 13] and the presence of antigens cross-reacting with other parasites [8, 15]. So, the definition of diagnostic antigens specific to *E. granulosus* has been expected for the development of practical serological tests.

As hydatid cyst fluid (HCF) contain many antigenic components, it seem to be a superior source as diagnostic antigen. As the basic research for development of serodiagnosis of hydatidosis, the present study was designed to compare the polypeptide components between hydatid cyst fluids obtained from cattle (HCFC) and sheep (HCFS), and to define antigenic components of HCFC and HCFS by immunoblot analysis.

II. Materials and Methods

1. Crude HCF and serum samples: Hydatid cysts were obtained from the lungs and livers of naturally infected cattle and sheep slaughtered at local abattoirs in Montevideo, Uruguay. HCF recovered from cysts was centrifuged as 9,000 rpm for 30 min. The supernatant was dialysed against H_2O, freeze-dried, and used as crude

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HCF.

Sera of cattle and sheep infected with and without hydatid cysts by macroscopic examination in abattoir were used as positive and negative sera, respectively.

2. SDS-PAGE and immunoblot: SDS-PAGE was performed according to the procedure of Laemmli [7]. Briefly, crude HCF was dissolved in SDS-sample buffer (0.05 M Tris-HCl pH 6.8, 10% SDS, 10% glycerol) with or without 2.5% 2-mercaptoethanol as a reductant, and then boiled at 100°C for 3 min. The crude HCF was run on 5-20% gradient gels at constant current of 25 mA. After electrophoresis, polypeptides separated on the gels were stained with coomasie briliant blue (CBB) or india ink, or blotted electrophoretically onto polyvinylidine difluoride membranes. The membrane was cut into 16 strips of 4 mm in width. The strips were incubated for 1 hr with PBS-Tween (PBS pH 7.2, 0.3% Tween-20) containing 2% gelatin, and then exposed for 1hr to positive or negative sera diluted 1 : 100 with PBS-Tween containing 0.5% gelatin. After washing several times in PBS-Tweens, the strips were incubated for 1hr with horseradish peroxidase conjugated anti-bovine IgG or anti-sheep IgG antibody. After washing in PBS-Tween, bands were visualised by 3-3′ diaminobenzidine substrate.

III. Results

1. Comparision of polypeptide components between HCFC and HCFS: A number of polypeptide bands over the complete range of molecular weight appeared in both HCFC and HCFS. Polypeptides of HCFS were separated dominantly into 3 bands of more than 200 kDa and 8 bands of 60 kDa, 58 kDa, 50 kDa, 34 kDa, 27 kDa, 21 kDa, 14 kDa, and 8 kDa under non-reducing condition, and into 8 bands of 96 kDa, 90 kDa, 66 kDa, 38 kDa, 27 kDa, 21 kDa, 14 kDa and 8 kDa under reducing condition. On the other hand, polypeptides of HCFC were composed of 7 dominant bands of >200 kDa, 108 kDa, 62 kDa, 60 kDa, 54 kDa, 14 kDa and 8 kDa under non-reducing condition, and of 6 major bands of 200 kDa, 62 kDa, 52 kDa, 30 kDa, 14 kDa and 8 kDa under reducing condition (Fig. 1).

2. Immunoblot analysis of antigenic components in HCFC and HCFS: Most of polypeptides in HCFC and HCFS were recognized by both positive and negative sera. However, polypeptides of 50 kDa, 27 kDa and 14 kDa in HCFS were recognized by 29, 23 and 25 sera out of 29 positive sera of cattle, respectively, but also by 3, 1 and 1 sera out of 9 negative sera of cattle, respectively (Fig. 2). Moreover, polypeptide bands of 21 kDa 14 kDa and 8 kDa in HCFC were detected by 17, 13 and 17 sera out of 25 positive sera of sheep, respectively, but also by a few negative sera (Fig. 5). On the other hand, when HCFS and serum samples of sheep, or HCFC and serum samples of cattle were tested by immunoblot, both positive and negative sera recognized the same polypeptides (Figs. 3 and 4).

IV. Discussion

The results of SDS-PAGE in the present study

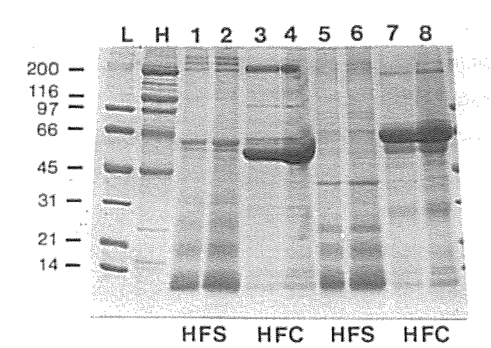


Fig. 1. Comparision of polypeptide components between HCFC and HCFS by SDS-PAGE.
Lanes 1 and 2, and 3 and 4 show HCFS and HCFC under non-reducing condition, respectively. Lanes 5 and 6, and 7 and 8 show HCFS and HCFC under reducing condition, respectively. Lanes L and H show marker protein (size in kDa is indicated).

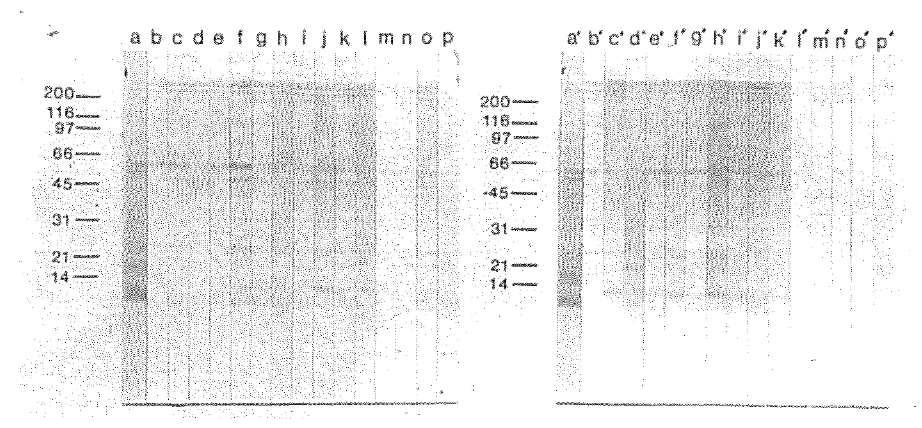


Fig. 2. Immunoblot of HCFS using sera of cattle infected with and without hydatid cysts. Lanes (b)-(l) and (b')-(k'), and (m)-(p) and (l')-(p') were incubated with sera with and without hydatid cysts, respectively. Lane (a) was stained with CBB for protein. HCFS was resolved in sample buffer without 2-mercaptoethanol. Size of marker protein is indicated in kDa.

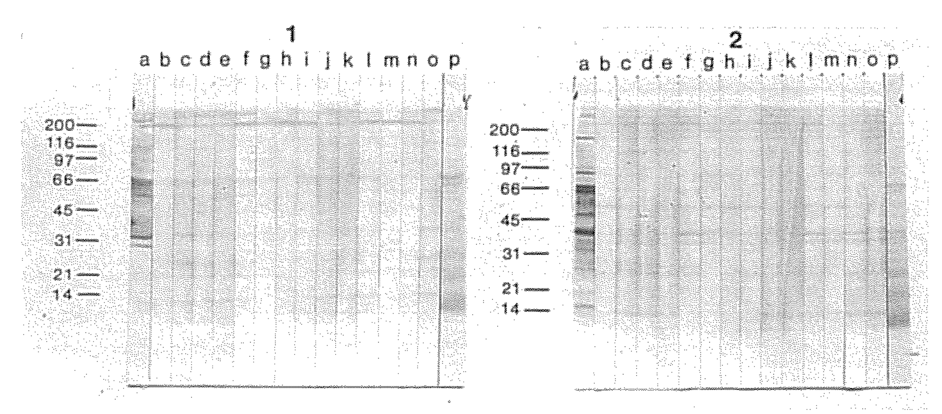


Fig. 3. Immunoblot of HCFS using sera of sheep infected with and without hydatid cysts. Lanes (b)-(k) and (l)-(o) were incubated with sera with and without hydatid cysts, respectively. Lanes (a) and (p) were stained with india ink and CBB, respectively. HCFS was resolved in sample buffer without (1) and with (2) 2-mercaptoethanol. Size of marker proteins is indicated in kDa.

have shown that HCFC and HCFS were different in dominant polypeptide components. Gottstein et al. [5] also demonstrated that polypeptide components of hydatid fluid isolated from various hosts differed from each other. However, it was reported that hydatid fluid was composed of not only parasite-derived, but host-derived components such as albumin, immunogloblin and various other serum components [2], and furthermore some host-derived components bound to IgG of serum sample and horseradish peroxidase conjugated anti-IgG antibody [9]. Moreover, the results of the present immunoblot analysis suggested that most of the dominant polypeptide components in HCFS and HCFC did not have antigenicity specific to hydatid infections. From respects described above, it was thought that most of HCF polypeptides was derived from host, and that these host-derived components caused the difference in dominant bands between HCFC and HCFS.

The two major antigens of HCF are called Antigen 5 and Antigen B [11]. Antigen 5 is a heat-labile lipoprotein composed of 24 kDa and 38

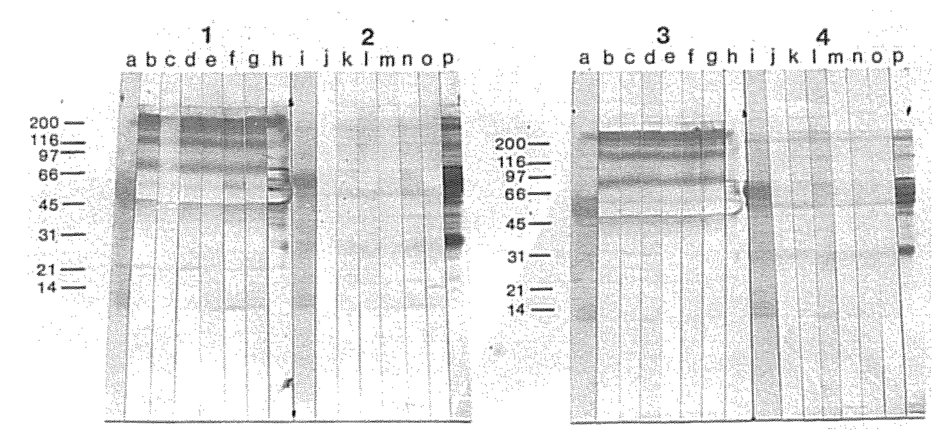


Fig. 4. Immunoblot of HCFC using sera of cattle infected with and without hydatid cysts. Lanes (b)-(e) and (j)-(m), and (f), (g), (n) and (o) were incubated with sera with and without hydatid cysts, respectivity. Lanes (a) and (i), and (h) and (p) were stained with CBB and india ink, respectively. HCFC was resolved in sample buffer without (1, 3) and with (2, 4) 2-mercaptoethanol. Size of marker protein is indicated in kDa.

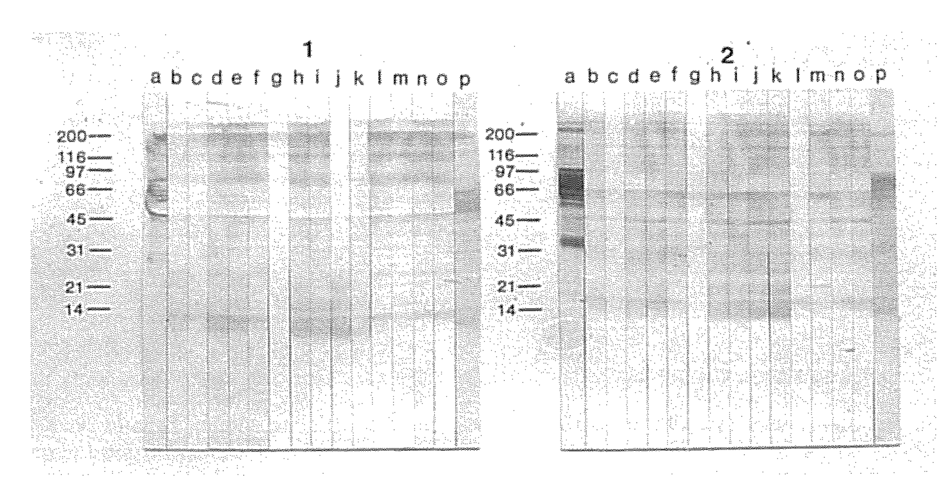


Fig. 5. Immunoblot of HCFC using sera of sheep infected with and without hydatid cysts. Lanes (b)-(k) and (l)-(o) were incubated with sera with and without hydatid cysts, respectively. Lane (a) and (p) were stained with india ink and CBB, respectively. HCFC was resolved in sample buffer without (1) and with (2) 2-mercaptoethanol. Size of marker proteins is indicated in kDa.

kDa subunits linked by disulphide bonding [10]. On the other hand, Antigen B in a heat -stable lipoprotein consisting of a regularly spaced group of molecules with the smallest subunit estimated to be 8 kDa and other components each differing in size by approximately 8 kDa [10]. Moreover, Stepherd and McManus [13] mentioned that dominant antigens of HCF were 3 subunits which were estimated to be 12 kDa, 16 kDa and 20 kDa and likely corresponded to Antigen B, and 2 subunits which were estimated to be 20 kDa and 38 kDa and corresponded to Antigen 5. In the present study, 5 polypeptides of 50 kDa, 27 kDa, 21 kDa, 14 kDa and 8 kDa in HCFC and HCFS were recognized by most of positive sera. We presumed that these polypeptides were corresponded to subunits of Antigen B, although molecular weight estimated in the present study did not agreed completely with that of Antigen B reported previously, since these polypeptides retained

antigenicity after boiling at 100°C for 3 min. However, these 5 polypeptides were recognized also by a few negative sera. With respect to the reaction, we assumed that the negative sera used had contained antibodies to hydatid cyst and/or to antigenically cross-reacting parasites such as *Taenia* spp. and *Fasciola hepatica* [4, 14], since we carried out only macroscopic examination in detection of infection with hydatid cysts and other parasites.

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単包虫感染牛及び羊の血清が認識する包虫液抗原の イムノブロット法による解析

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板垣　匡・坂本　司・Patricia BERASAIN・Jaqueline MAISONNAVE・Luis YARZÁBAL (1994) **単包虫感染牛及び羊の血清が認識する包虫液抗原のイムノブロット法による解析　岩手大農報　22 : 25-30.**
単包虫感染牛及び羊から得た包虫液（HCFC, HCFS）をSDS-PAGEで分画後，単包虫感染牛及び羊血清との反応性をイムノブロット法により調べた．SDS-PAGEによりHCFCとHCFSの主要なポリペプチド構成はそれぞれ異なっていることが分かった．HCFCにおける推定分子量21 kDa, 14 kDaおよび8 kDaのポリペプチド，またHCFSにおける50 kDa, 27 kDaおよび14 kDaのポリペプチドはそれぞれ多数の単包虫感染羊または牛の血清に認識された．しかし，これらのポリペプチドは少数の単包虫非感染羊または牛の血清とも反応した．これらのポリペプチドは耐熱性であったことから，包虫液の主要抗原として知られているAntigen Bのサブユニットに相当すると考えられた．

キーワード：イムノブロット，*Echinococcus granulosus*, 家畜，単包虫，包虫液抗原．